

15

FIGURE 1

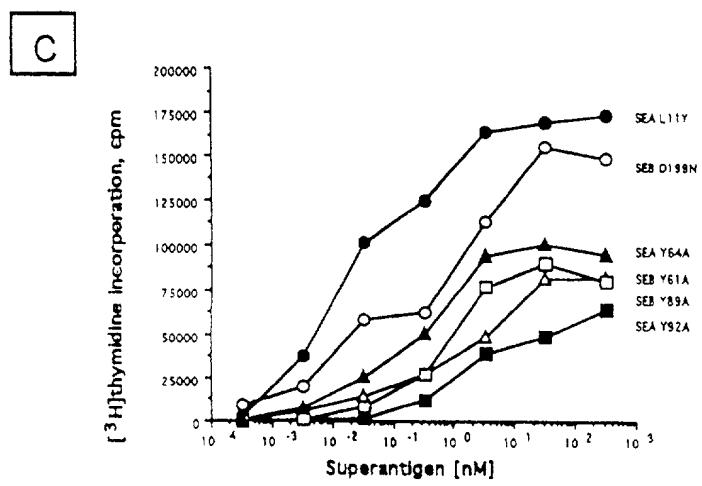
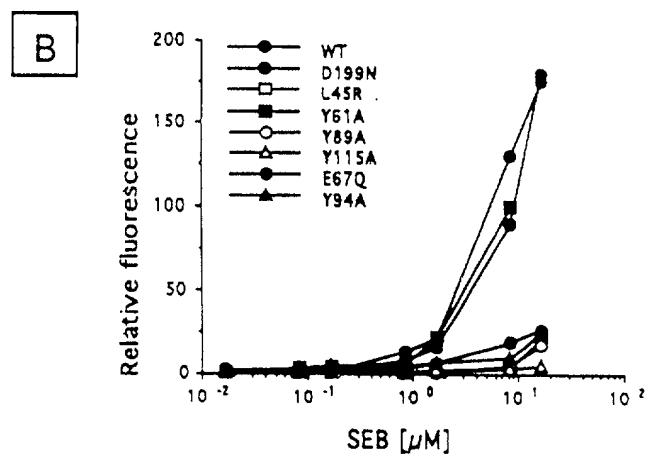
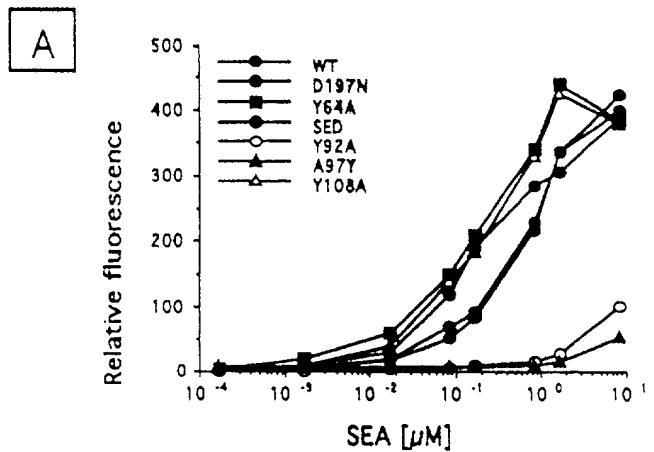


Fig. 2

48	70	92	108
SEA . . . SHDQF [QHTTILFKGFFTDHSWYNDLLVFDSDKDILVDKYK . GKKVLDLYGAY [GYQCA CGTPNKTACM [GGVTLLHDNNNRRLTEKK	TGDOF [ENTLLYRKFFFTDLINFEDELLI FNSKEMAQHFK . SRNVDVYPIR [SINCY GGELIDRTACT [GGYTPHEGNKLKERKK	SDDQF [ENTLLFKGFFFTGHPWYNDLLV LGSKDATNKYK . GKKVLDLYGAY [GYQCA GGTPNKTACM [GGVTLLHDNNNRRLTEKK	SIDQF [YFDLIYSIKDTKLGNYTDNVRVFKNKDIADKYK . DKYVUDVFGAN [YYQCYFSKKTNIDINSHQTDKRKT . CM [GGVTEIGNQLD . . KY
SEB . . . SEC1 . . . SVDKF [AHDLIYNI SDKKLKNYDKVKT [LNNEGIAKKYK . DEVVDVYGSN [YVNCYFSSKDNYGVTVGG . . . KT . CM [GGITKHEGNHF DONGNL	SEC2 . . . SEC3 . . . SVDKF [AHDLIYNI SDKKLKNYDKVKT [LNNEGLAKKYK . DEVVDVYGSN [YVNCYFSSKDNYGVTVGG . . . KT . CM [GGITKHEGNHF DONGNL	SPEa . . . SVDQI . . . SVDNF [SHDLIYNVSG . . . PNYDKLKT [LNQEMATLFK . DRNVDIYGVE [YHLCYLCENAE RSACI [GGVTNHEGNHLEIPK.	TSST1 . . . VLDNS [GSMRKNTD . . . GSISLII FPSPYYSPAFTKGKEVKDLNTKR [KKSQHTSEG TYIHFIHF . Q [SGVTNT EKLPT . . . P

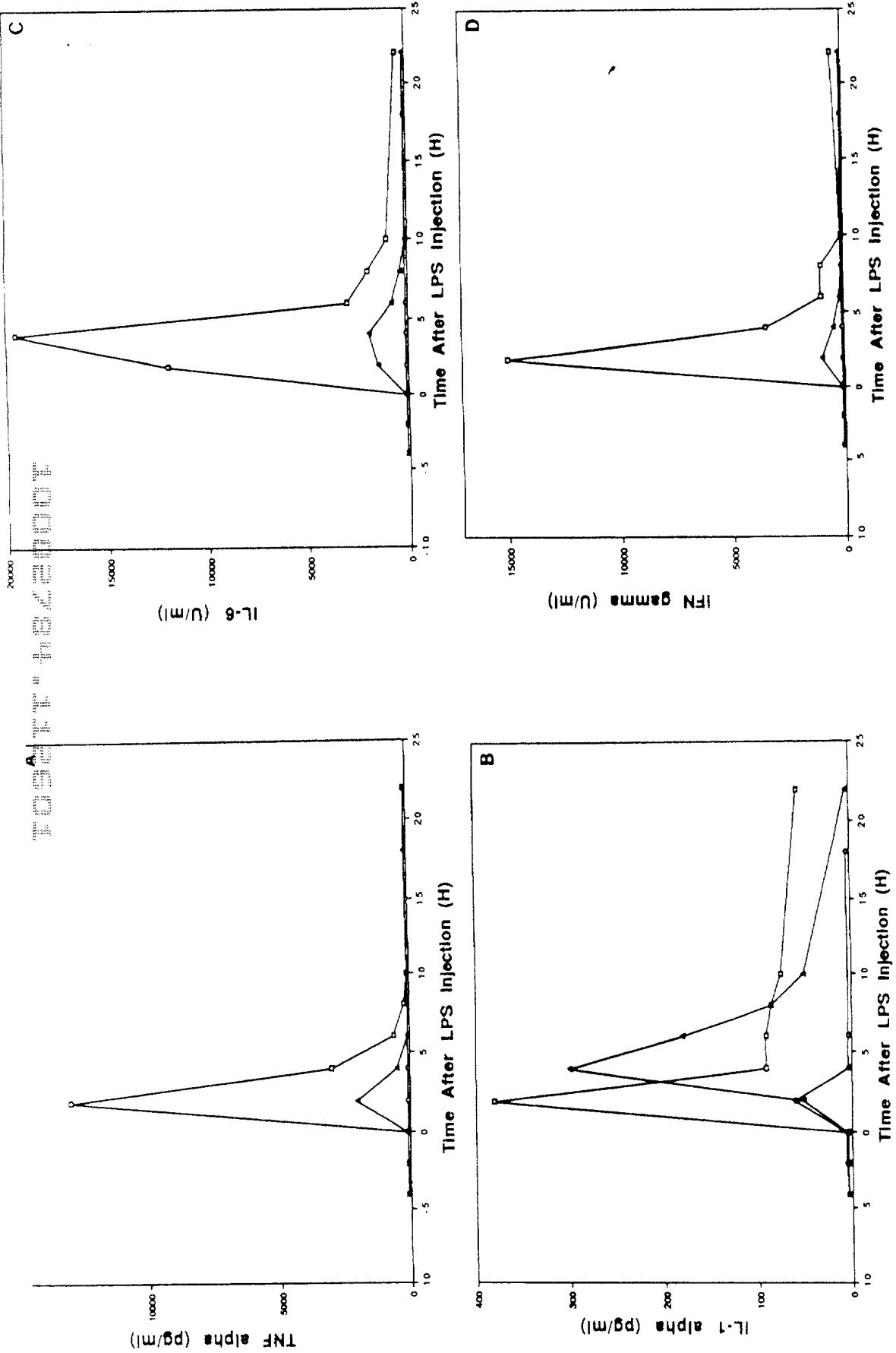


Fig 4

WT-SEA SEA K14E SEA Y64A SEA Y92A Adjuvant Untreated

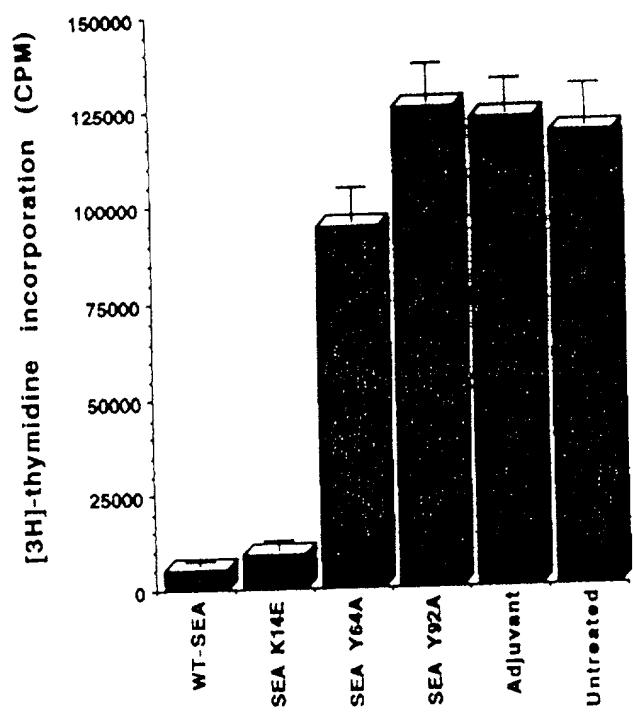


Fig. 5

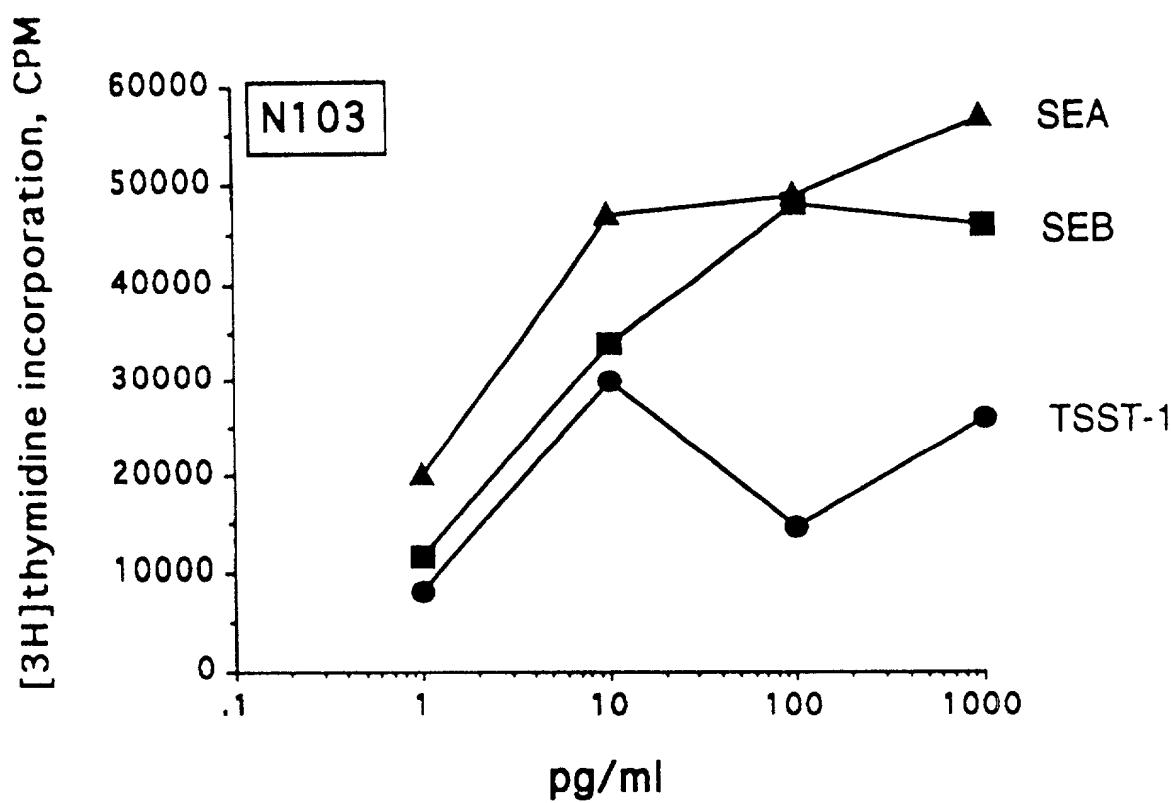
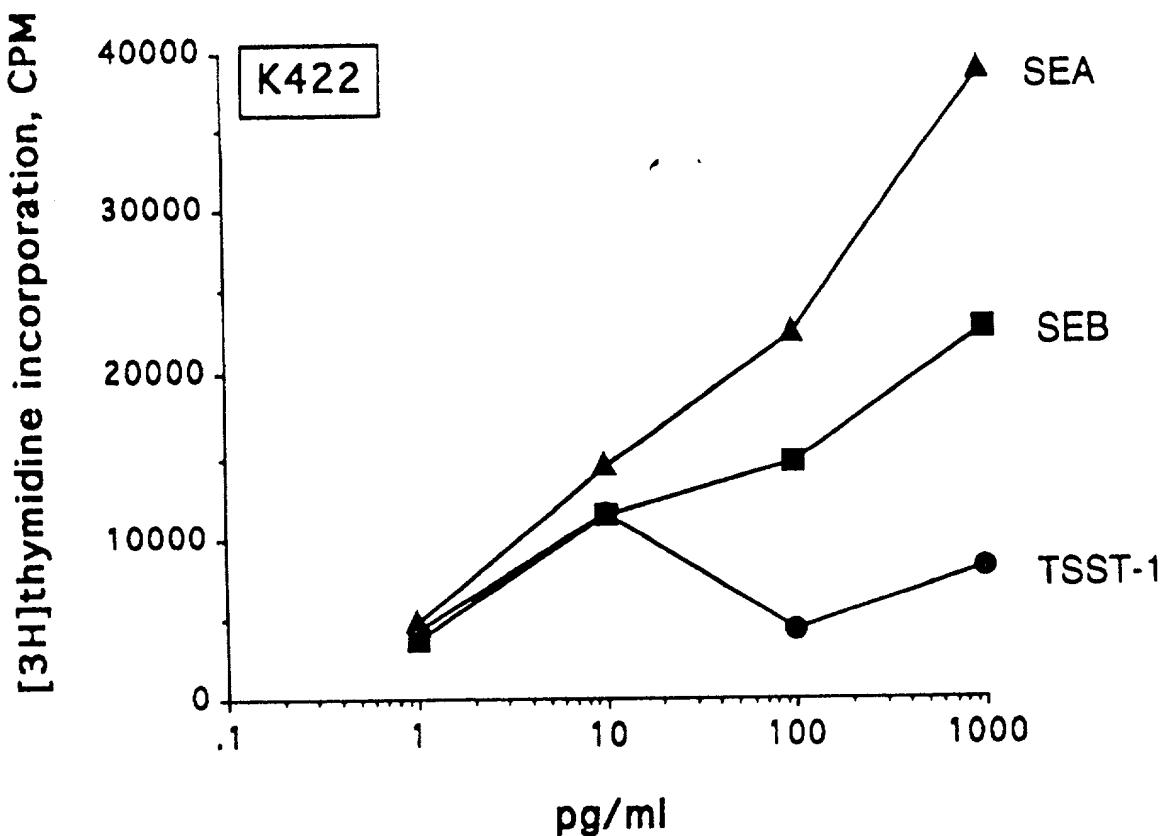
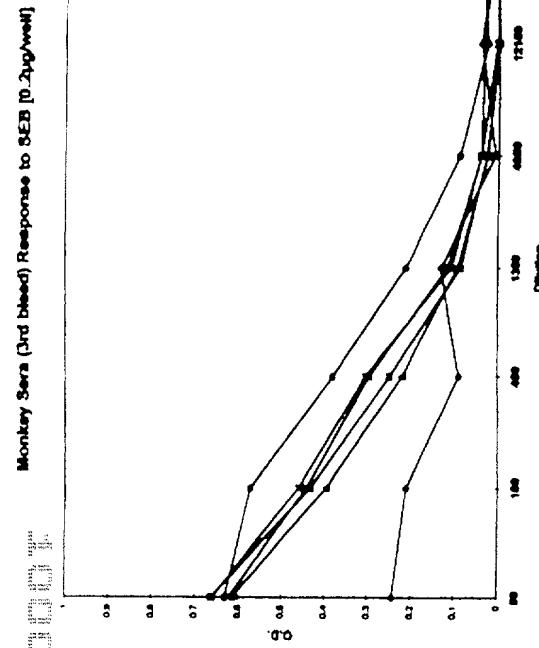
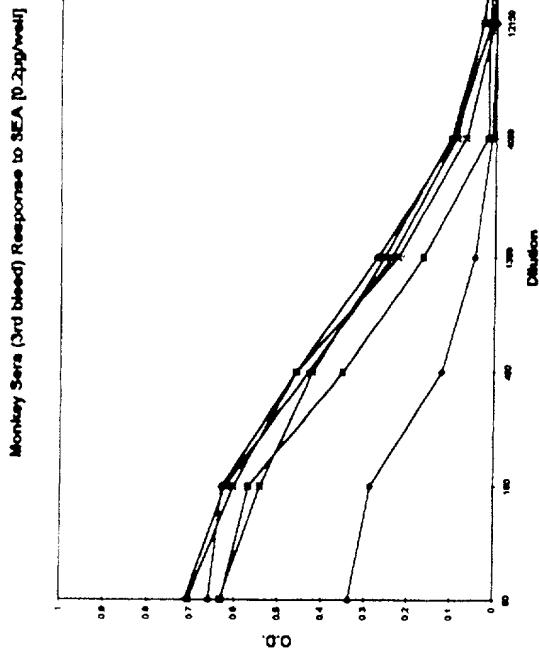


Fig. 6



Monkey Sera (3rd blood) Response to SEC [D-25g/mm].

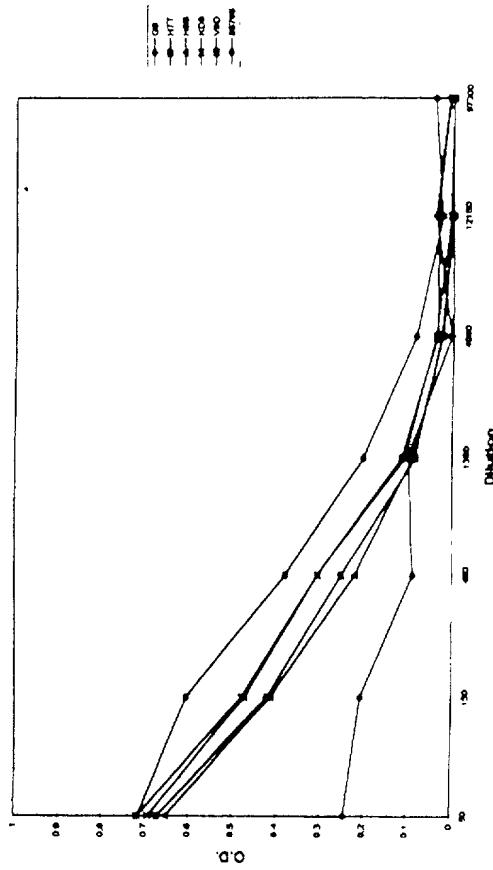
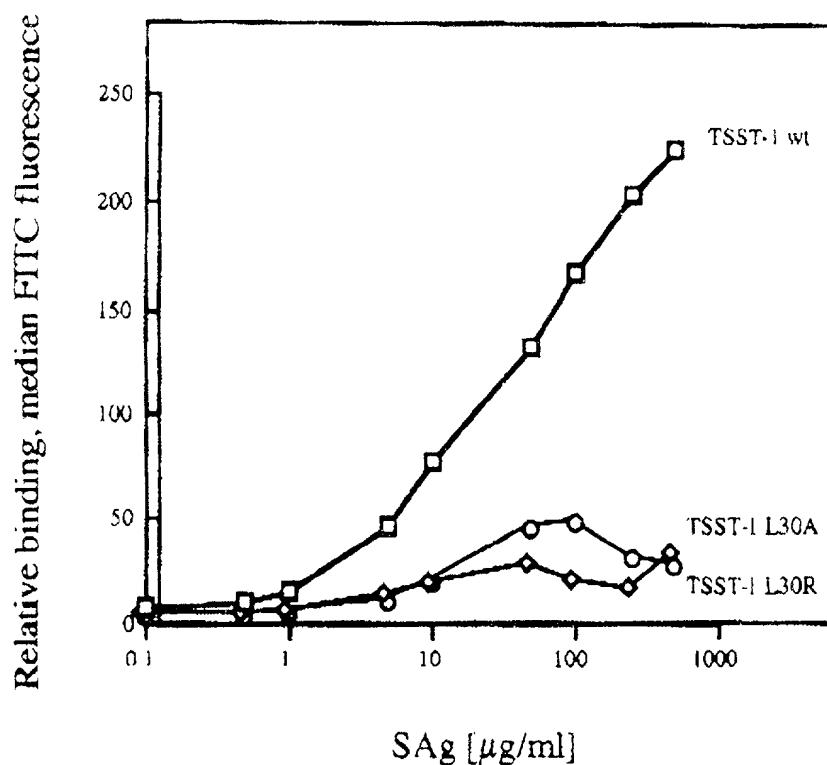


Fig. 7

A.



B.

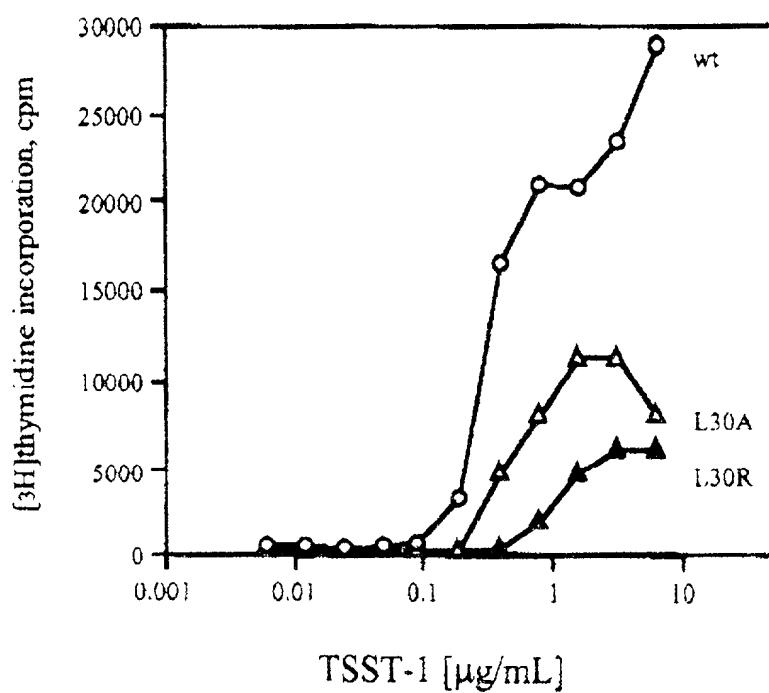
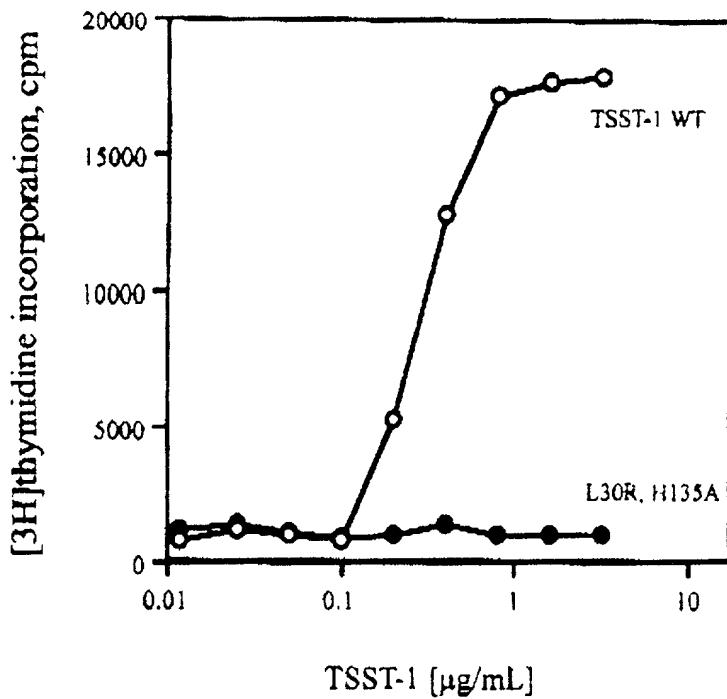


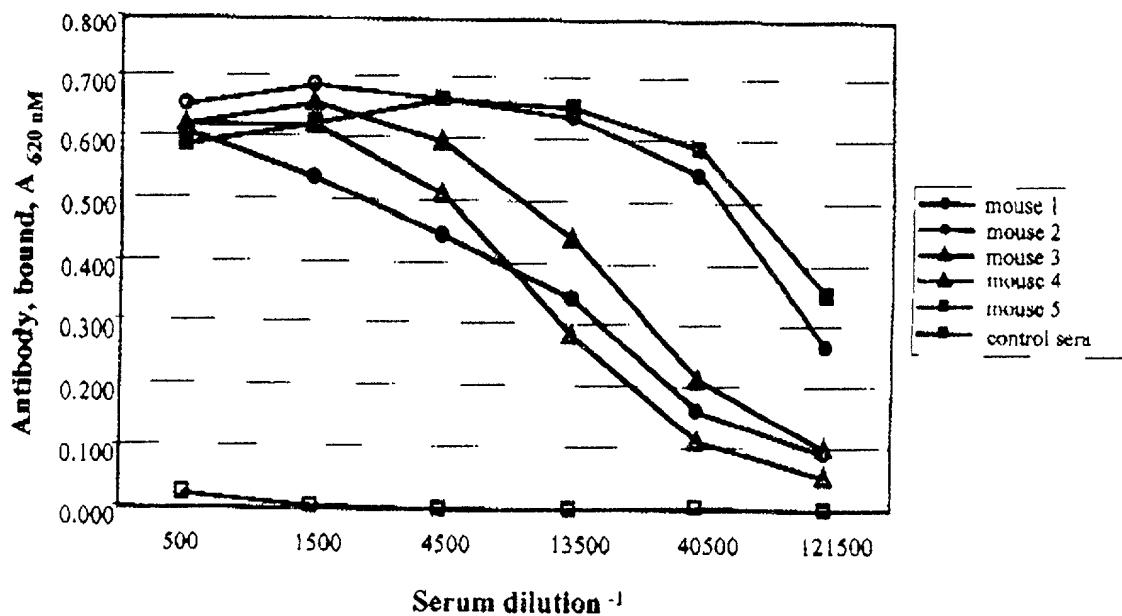
FIGURE 8

C.



Biological activities of TSST-1 mutants. a, Mutations of TSST-1 at amino acid position 30 (L30R, L30A) results in greatly diminished interactions with cell surface HLA-DR, measured by laser fluorescence-activated flow cytometry and FITC-labeled rabbit anti-TSST-1 antibody (affinity purified). b, Mutations of TSST-1 at amino acid position 30 (L30R, L30A) results in greatly diminished activation of human lymphocytes; c, Introduction of an additional mutation, H135A to the TSST-1 mutant L30R results in the maximum reduction in T-cell stimulation. Human T-cell proliferation, was assessed by $[^{3}\text{H}]$ thymidine incorporation, using a 12 h pulse with label and harvesting cells after 60 h of culture. Each data point represents the mean of triplicate determinations; SEM <5%.

FIGURE 8



Antibody response to TSST-1 mutant L30R. Mice received a total of three injections of vaccine ($20 \mu\text{g}/\text{mouse}$) in Alhydrogel, two weeks between injections. Sera were sampled two weeks after last vaccination and anti-TSST-1 specific antibody was measured by ELISA, using plates coated with wild-type TSST-1. Pooled non-immune mouse sera were used as negative control.

FIGURE 9

A.

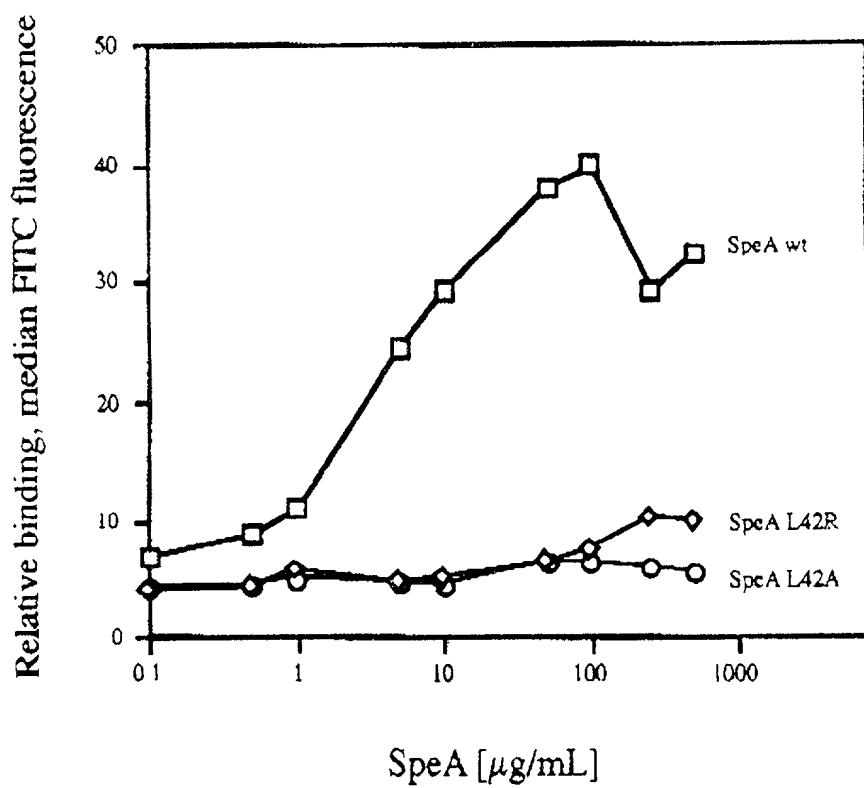
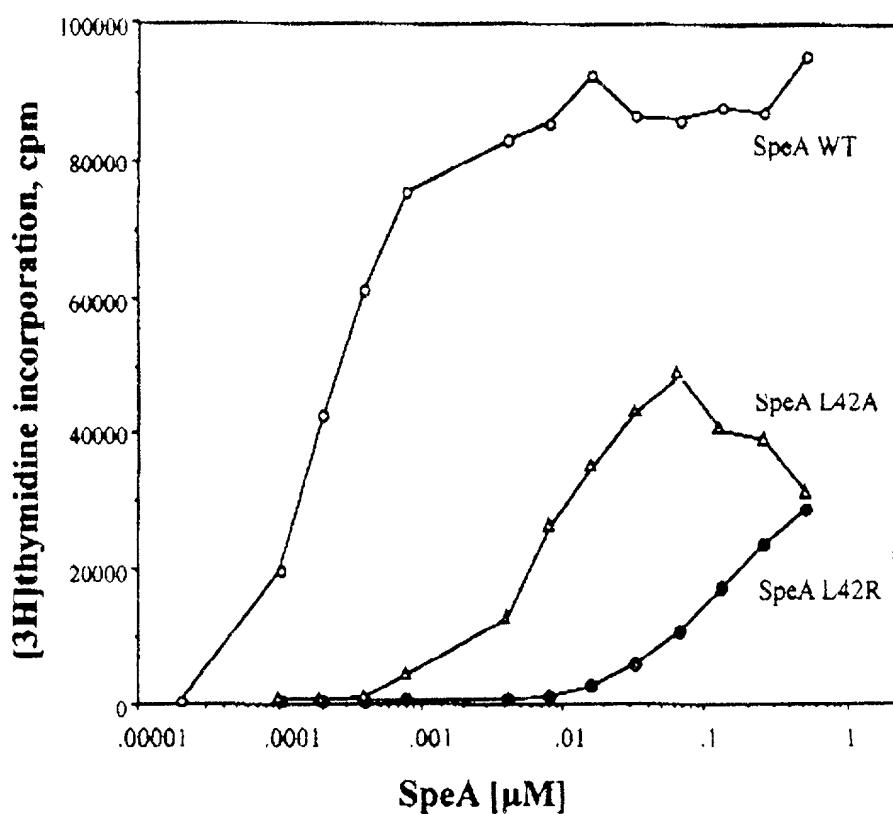


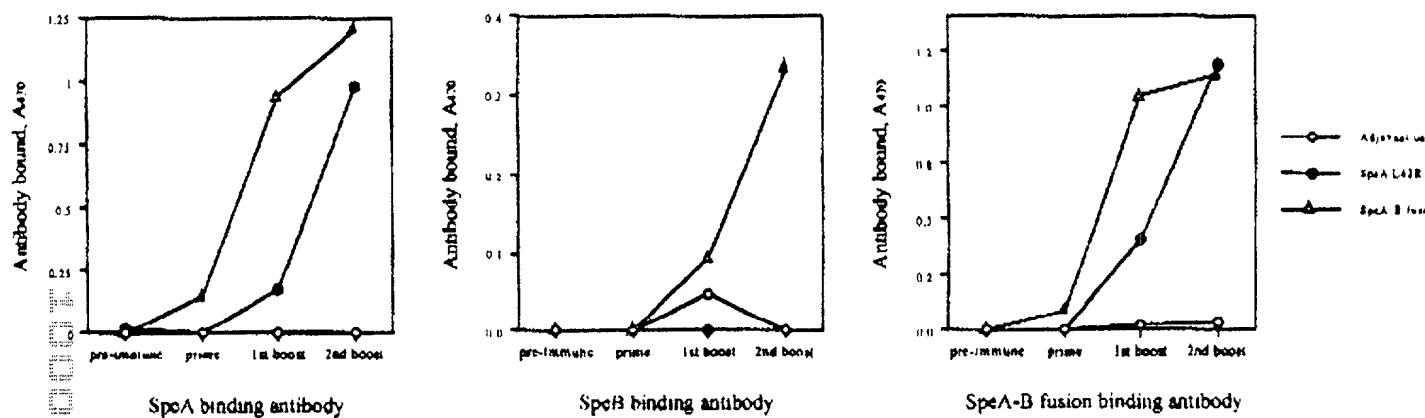
FIGURE 10

B.



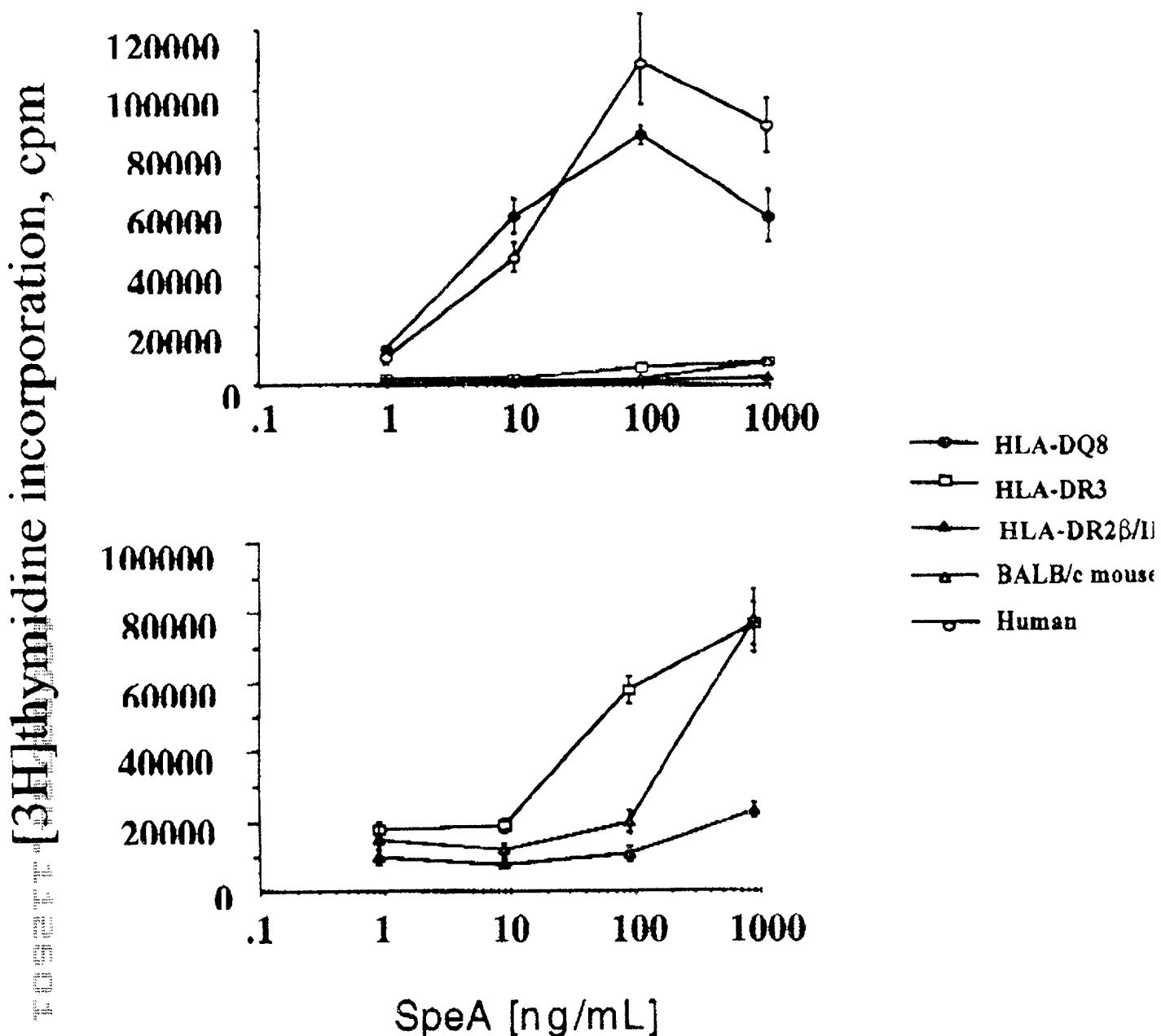
Biological activities of SpeA mutants. a, Mutations of SpeA at amino acid position 42 (L42R) results in greatly diminished interactions with cell surface HLA-DR, measured by laser fluorescence-activated flow cytometry and FITC-labeled rabbit anti-SpeA antibody (affinity purified). b Mutations of SpeA at amino acid position 42 (L42R or L42A) results in greatly diminished activation of human lymphocytes. Human T-cell proliferation, was assessed by $[3^H]$ thymidine incorporation, using a 12 h pulse with label and harvesting cells after 60 h of culture. Each data point represents the mean of triplicate determinations; SEM <5%.

FIGURE 10



Mouse antibody response to SpeA L42R and SpeA-B fusion constructs. BALB/c mice were vaccinated three times with 10 µg plus adjuvant (MPL™ + TDM+ CWS Emulsion, RIBI ImmunoCHem Research, Inc., Hamilton, MT) of each construct, allowing two weeks between injections. Sera from each experimental group ($n=5$) were pooled for measurement of specific antibodies. Data shown are antigen-specific antibodies (ELISA units) present in a 1:100,000 dilution of pooled sera from mice vaccinated with SpeA L42R, SpeA-B fusion or adjuvant only.

FIGURE 11



T-cell response *in vitro* of mononuclear cells from transgenic mice expressing HLA-DQ8 $\alpha\beta$ and human CD4 closely approximate the physiological response of humans. Mononuclear cells were isolated from spleens of transgenic mice expressing HLA-DR3, HLA-DQ8 or HLA-DR2 β /IE α , or non-transgenic BALB/c mice and human peripheral blood (4×10^5 /well). Following 60 h culture with SpeA, cells were pulse-labeled (12 h) with 1 μ Ci of [³H]thymidine. DNA from cells was harvested onto fiberglass filters and incorporated radioactivity measured by liquid scintillation.

FIGURE 12